Seascape®-BWMS Shipboard Testing

The Implementation Plan of Sample Measurement and Quality Control

The First Institute of Oceanography, State Oceanic Administration

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Contents

1. Trial Objective

Ballast water is used widely to maintain the stability and maneuverability of ships during transit. However, the transport of ballast water causes introduction of unwanted organisms and the resultant damage to ecological, which is regarded as one of the four major risk factors that threaten global marine environmental safety. In June 1997, a poisonous Marine dinoflagellate - *Alexandrium* that could usually be seen in the coast of The North Sea, the Atlantic ocean, the Mediterranean, the east coast of the US, Australia, Japan, New Zealand leaded algal bloom in the coastal waters of Norway and England where it should not appear. The possible reason was that ballast water brought the algae to the area. Based on this international threat, governments around the world pay more attention to ballast water management. The International Maritime Organization and the International Environmental Protection Organization successively formulated international conventions to prevent and control marine pollution. The International Conference on Ballast Water Management for Ships held in February 2004 adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 together with four conference resolutions. Some marine scientific research departments and enterprises have developed several ballast water treatment methods, instruments and devices since 2008. Seascape® - BWMS developed by Elite Marine Ballast Water Management System Corp. uses UV＆US processing technology, and has gone into testing stage in the laboratory.

Entrusted by Elite Marine Ballast Water Management System Corp., we tested the efficiency of Seascape®-BWMS, including the water quality and marine organisms during the testing stage in the laboratory.

2. Testing content

According to the requirements of the project and G8 guidelines, the following parameters should be tested:

1) Environmental parameters:

Temperature, Salinity, TSS, POC.

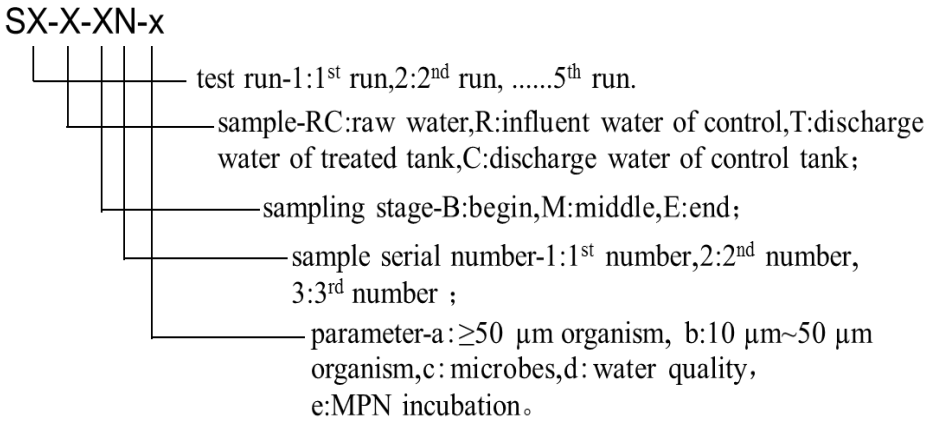
2) Organisms:

- Organisms with size greater than 50 μm;
- Organisms with size between 10 and 50 μm;
- Heterotrophic bacteria and human pathogens (*Escherichia coli*, Intestinal *Enterococci* and *Vibrio cholera*).
- Chlorophyll-based most probable number(MPN) determination

3. The methods of sampling, storage, transport and analysis of samples

3.1 Sample numbering

All sample bottles should be attached a tag. Numbering method of sample tag is as follows:



3.2 Collection of samples

3.2.1 Collection of water quality samples

1) Temperature and Salinity:

A multi-parameter water quality instrument is used to measure the water temperature and salinity immediately after each water sample is collected in sampling container. Data of water temperature and salinity is recorded after the value on the screen is stable.

2) TSS:

2.5 L water sample is collected into clean plastic bottles.

3）POC:

500 mL water sample is collected into clean glass bottles. During sampling process, analyzers should wear clean gloves.

3.2.2 Collection of organism sample:

1) Organisms with sizes greater than 50 μm

For raw water samples, treated water samples and control water samples during uptake and discharge, 1 m^3 water sample is to be collected each sample. Sampling time is to last for longer than 90 s. While sampling, flow rate is controlled to be no more than 40m^3/h and ensure the nets to be submerged in the water.

Water samples are filtered through 50 μm mesh (in the diagonal dimension) nylon net and concentrated to 100 mL-200 mL, and then transfer into a new small

bottle with label.

2) Organisms with size between 10 μm and 50 μm:

All water samples will be filtered through 10 μm mesh (in the diagonal dimension) nylon net. 1 L water sample is quantitated by a PPCO and concentrated to 100 mL-200 mL, and then shifted into a new small bottle with label.

3) Microbes

The Samples for microbe analysis must be collected with sterile operation. Sample bottles were treated with high temperature sterilization. Each water sample need 500 mL.

4) MPN water samples

Water samples will be collected into clear plastic bottle of 1.2 L which is pre-cleaned by dilute HCl and then washed several times with pure water.

3.3 Storage and transportation of the samples

3.3.1 Water quality samples

1) TSS:

Water samples are pre-treated in the field laboratory on ship board.

i) Pre-treatment: Pre-weighted and coded acetates fiber membrane is used to filter water sample. The filtered volume was dependent on the particle matter and concentration and type of organisms present in the water. The higher the total particle matter in the sample, the smaller was the volume that could be filtered before the filter clogs. Practical volumes were between 100 mL and 1000 mL per sample, after filtration the filter was rinsed with fresh water (Mili Q) to remove sea salt. After filtration, each fiber membrane is folded and packed by aluminum foil. Then they are put in sealed plastic bag and wrote sample number outside the plastic bag and put into freezer on the board to freeze.

ii) Storage and transportation: Sealed plastic bags mentioned above are frozen on board, and then transported to laboratory in Qingdao in ice box.

2）POC:

Water samples are pre-treated in field laboratory on board.

i) Pre-treating: Water samples were filtered over a glass fiber membrane (GF/F) combusted under 450 ℃ in muffle furnace. The filtered volume depends on the particle concentration and abundance of organisms present in the water. After filtration, each fiber membrane is folded and packed by aluminum foil. Then they are numbered and put in sealed plastic bags.

ii) Storage and transportation: Sealed plastic bags mentioned above are stored in freezing on board and transported to laboratory in Qingdao in ice box.

During storage and transportation of TSS and POC samples, the freezer is designated for these samples and no other material is allowed to be put inside.

Conspicuous mark is put on the freezer to avoid any accidents that might affect the testing's availability.

If the samples of TSS and POC can be not measured immediately after are transited to our lab, the samples stored in ice-freezer again and waiting for analysis at the end of each testing stage. Another stored method is that the samples on filters for TSS and POC were dried 12 h at 60 ℃ and allowed to cool to ambient temperature, and should be moved to petri dish and put into a vacuum desiccator until measure to be taken.

3.3.2 Storage of samples for biological analysis

For the inflow ballast water samples, all the organisms were assumed to be viable. After the sampling, organisms ≥50 μm were fixed with formalin (final concentration 4%), and organisms 10 μm~50 μm were fixed with Lugol's solution (final concentration 2%), and stored under the ambient temperature.

For the biological samples in the discharge waters, organism ≥50 μm were dyed with neutral red and stored under the ambient temperature. The organisms ≥10 μm~50 μm without adding fixing agent were stored in the incubator and put into ice bags. The microbe samples are put in plastic box under the low temperature.

3.4 Analysis method of samples

3.4.1 Environmental parameters:

1) Temperature and Salinity: After each water sample is collected into sampling container temperature and salinity are measured directly by a probe on a multi parameter water quality instrument.

2) TSS：Weight method. Filters were dried overnight at 60 ℃ and allowed to cool in a vacuum desiccator before weighting. The total amount of suspended solids was calculated from the weight increase of the filter.

3) POC：The high temperature combustion method. Measured with CHN elemental analyzer. The samples on filters were put in aluminum foil, dried over 12 h at 60 ℃. The CHN elemental analyzer (Elementar Vario ELIII, made in German) was used to measure POC. If the samples are not measured immediately, dried samples on filters should be transfered to petri dish and put into a glass dryer until measure to be taken.

3.4.2 Organisms samples:

1) Organisms with sizes greater than 50 μm:

Identification and counting of viable organisms were taken with a stereo microscope.

i) Neutral Red dye

In order to investigate and count the live and dead organism ≥ 50 μm, neutral red stains living organisms distinctively and quite rapidly. Therefore the viability

assessment remains unaffected by the possible death of organisms during the staining or during sample analysis. It is assumed that dead but physically intact organisms will also be found. Consequently a detailed inspection of each intact individual is needed to assess viability. This includes the staining as well as the detection of internal (heart, gills) movement. Organisms which were not intact are assumed to be dead. Neutral Red is a reliable staining method for all major groups of organisms but inconsistent staining was found for bivalves. For this latter group movement (including internal such as heart and gills for juvenile mussels) has to be used obligatorily to determine viability. The neutral red is added to the concentrated sample with an end concentration of 1:50,000. Staining time is 2 hours. Before analysis, the dye of body surface of organism should be washed clean with filtered sea water or fresh water.

ii) Splitting sample

For influent water and discharge control water, the sample needs to split if density of organism is high. The sample is divided into two "equal" portions using a plankton splitter. One subsample from the split is saved in a labeled jar indicating the fraction of total original volume it contains (½). The second subsample from the split is placed in the plankton splitter and divided again. One subsample is saved in a labeled jar indicating the fraction of the total original volume it contains (¼).

Repeat the above steps as many times as necessary until the last two subsamples contain at least 200 and no more than 400 individuals. These two subsamples of equal fraction are saved in appropriately labeled jars.

For treated samples of discharge water for treated tank, there is no need to split. The sample should be counted totally by microscopy.

iii) Microscopic counting

Three subsamples are to be examined and enumerated by stereo microscopy.

Remove the aqueous portion of the subsample with the condensing tube and transfer the remaining organisms to the zooplankton chamber.

Scan the whole zooplankton chamber in low magnification about 20× to observe the distribution of the organisms.

Count and identify of the organism under the stereo microscopy. For enumeration, 50×magnification is ok. For identification, about 100×magnification is preferred. Continue count, until the minimum number 200 individuals.

iv) Calculation of abundance

The number of individuals per cubic metre was calculated as follow equation.

$$C_B = \frac{N_B}{V}$$

Where:

C_B — density of plankton, unit: ind./m^3;

N_B — quantity of plankton filtered by net, unit: individuals or cells;

V — volume of filtered ballast water, unit: m^3.

iii) Storage:

When the counting of viable organisms was finished, samples are fixed with formaldehyde solution (the last concentration is 5%) and stored for several months at least so as to future recheck.

2) Organisms with size between 10 and 50 μm:

- **For raw and influent water samples:**

i) preparation of counting sample

①The samples are fixed with Lugol's iodine solution of final concentration 2%.

②The volume of concentrated samples should be measured before counting. If the density of sample is very high or low, it may be necessary to dilute the sample.

③Just before taking a sub-sample, the sample must be thoroughly mixed. The sample should be mixed using a combination of alternating horizontal rolling and vertical tumbling (turning upside and down) of the sample bottle for 2 minutes. These actions should be gentle and not involved any vigorous shaking.

④After thoroughly mixing, using a pipette and clean tip, 1 mL（or 0.5mL） of the sample as a subsample. For the same sample, three sub-samples are taken and counted by inverted microscope.

ii) Counting and identification:

① Place a 1 mL(or 0.5 mL) Sedgewick-Rafter counting chamber on a clean paper towel or kim wipe to avoid scratching the bottom surface. Using a pipette and clean tip, dispense 1 mL of the sample onto the Sedgewick-Rafter counting chamber. If the density is high, 0.5 mL chamber is used alternatively. For the same sample, three sub-samples are taken and counted by microscope.

②Slide a cover slip in place, assuring no air bubbles are trapped in the slide. If there are air bubbles, they can often be removed if you lightly maneuver the cover glass from side-to-side. If this does not work, reload the slide with a new sample.

③Once the slide is filled, let settle for approximately 5-10 minutes to allow the plankton to settle into a single layer. This makes counting easier. Use this settling time to calibrate the scale on the ocular micrometer.

④Put the chamber under the fluorescence microscopy. Set the microscope to its lowest focus and bring the sample into focus. Be sure focus on the sample in the side

and not on top of the coverslip.

⑤Change the focus to the next strongest magnification and bring the sample into focus. You will count only one transect of the Sedgewick-Rafter. Choose a random transect near the top or bottom of the slide. Methodically move through the slide identifying each cell you encounter. To avoid counting single plankton more than once, do not count phytoplankton that cross the bottom of the field of view. Continue counting full transects until you have a minimum of 300 plankton counted. Record the number of transects counted on the log sheet.

⑥For enumeration, 200× magnification is ok. For identification, 400× magnification is preferred.

The counting results are recorded on shipboard. All sample of this size are finished on shipboard in 6 hours.

- **For treated and control water samples in discharge:**

All samples for treated and control water samples in discharge will be measured after return the laboratory and finished in 24 hours.

Samples will be analyzed by following two methods:

i) FDA-PI stain:

The viability of organisms is assessed by staining with fluorescein diacetate (FDA) and propidium iodide (PI). FDA diffuses across cells with intact membranes due to esterase activity. Once entered into the active cells, the FDA substrate is cleaved by non-specific esterase releasing a polar fluorescein product that is retained inside cells with an intact membrane and the cells are stained green. The FDA can be excited at maximum 493nm and emits at 510 nm. Propidium iodide (PI) is a polar substance that easily penetrates only inactive or damaged cell membranes. Once inside the cell, PI binds to double strand nucleic acids with intercalation and fluorescence bright red under blue light excitation. The PI can be excited at maximum 540 nm and emits at 625 nm.

① Preparation of FDA-PI stored solution

A stored solution of fluorescein diacetate is prepared by dissolving in acetone with concentration of 5 mg/mL. The FDA working solution is freshly prepared by adding 0.04 mL of stock to 10mL of Dulbecco's phosphate buffered saline (DPBS). 1 mg of propidium iodide (PI) is dissolved in 50 mL of DPBS. The stock solutions of both dyes are stable up to 6 months when stored in the dark at 4 ℃. To stain with FDA-PI, FDA working solution and PI working solution are added to 10 mL subsample. The final concentration of FDA is 100μg/mL and the concentration of PI is 60 μg/mL. The cells are stained for 3 min at room temperature and then placed in fridge at 4 ℃ in dark before counting.

② Preparation of sub-sample

Dispense 10 mL of the sample to a tube as a subsample. For the same sample, three sub-samples are taken and counted by inverted fluorescence microscope.

③ Counting and identification:

a. swirl to mix the subsample.

b. Place a 1 mL(or 0.5 mL) Sedgewick-Rafter counting chamber on a clean paper towel or kim wipe to avoid scratching the bottom surface. Using a pipette and clean tip, dispense 1 mL of the sample onto the Sedgewick-Rafter counting chamber. If the density is high, 0.5 mL chamber is used alternatively. For the same sample, three sub-samples are taken and counted by microscope.

c. Slide a cover slip in place, assuring no air bubbles are trapped in the slide. If there are air bubbles, they can often be removed if you lightly maneuver the cover glass from side-to-side. If this does not work, reload the slide with a new sample.

d. Once the slide is filled, let settle for approximately 5-10 minutes to allow the plankton to settle into a single layer. This makes counting easier. Use this settling time to calibrate the scale on the ocular micrometer.

e. Put the chamber under the fluorescence microscopy. Set the microscope to its lowest focus and bring the sample into focus. Be sure focus on the sample in the side and not on top of the coverslip.

f. Change the focus to the next strongest magnification and bring the sample into focus. You will count only one transect of the Sedgewick-Rafter. Choose a random transect near the top or bottom of the slide. Methodically move through the slide identifying each cell you encounter. To avoid counting single plankton more than once, do not count phytoplankton that crosses the bottom of the field of view. Continue counting full transects until you have a minimum of 300 plankton counted. Record the number of transects counted on the log sheet.

g. For enumeration, 200× Magnification is ok. For identification, 400× Magnification is preferred.

h. For enumeration the vital and dead organism, flurorescence light source is used. For identification, normal light source is used.

④ Calculation of density of cells

$$C = \frac{n \bullet V_1}{V_2 \bullet V_n}$$

where：

C——density of organisms (cells/mL or ind./mL);

n——organisms number in one counting unit (cells or individuals);

V_1——sample volume after concentrated, unit (mL);

V_2——sample filtered over small sieve, unit (L);

V_n——sample volume for counting, unit (mL).

ii) MPN method:

Water samples can't be pre-treated and stored in freezer at about 4 ℃ and transported to laboratory in Qingdao, as well finished to culture within 24 h.

① Cultivation:

Each water sample is distributed to 500 mL conical flasks; f/2 culture media is added to each flask which will be then cultured in illumination incubator at 20 ℃. Photoperiod is 12h: 12h; culture period is 7~14 d. Two replicated samples are done in the testing.

Sampling frequency is:24 h, 48 h, 72 h, 96 h, 120 h, 144h, 168 h, 192 h, 240 h,288 h,may also be 336 h.

② Analysis method:

a) Sampling

Before sampling for detection, cultured water sample in conical flasks should be mixed adequately and take out more than 10 mL sample for detection

b) Detecting in vivo fluorescence

Chlorophyll fluorescence of live organisms of 10 mL culture water sample is detected daily by Turner Designs fluorometer. Value of *in vivo* fluorescence is used to indicate recover condition of phytoplankton.

c) Microscope detecting

0.5 mL culture water sample will be put in counting chamber and then detected with an inverted microscope. Conditions of cells is recorded which will be regarded as a supplementary method of fluorescence detecting method.

3) Microbe and human pathogens

i) Pre-treating:

No pre-treating is done for this testing process.

ii) Inoculation:

All samples are inoculated within 2 h after sampling and cultured in incubator on board. On shipboard, there will be 3 different incubators to make sure that 3 temperature conditions (25 ℃, 37 ℃ and 44 ℃) can be precisely kept. After inoculation, each culture dish will be placed in relevant incubator for culturing. All culture and counting work will be done in field laboratory on shipboard. During each bacteria analysis process, blank control tests will be done.

iii) Analysis:

a) *Heterotrophic bacteria*: plate counting method

Principles: After incubation of a sample, the dispersed bacteria are developed into isolated colonies. A visible colony on solid medium represents one bacterial cell. The number of heterotrophic bacteria is obtained by counting the number of colonies. The key of this technique is to disperse the heterotrophic bacteria completely and to dilute

bacterial sample to several solutions with different concentration. Small volume of diluted solution is spread evenly over the surface of the solid medium.

Procedures: For raw water samples and control water samples, 1 mL Tween solution is added to 100 mL sample. The sample is then well mixed to separate the organisms and kept separately. For treated water samples, no Tween solution is added to samples. 0.1 mL of above sample is inoculated on the surface of solid medium (2216E). Then it is spread evenly with a sterile glass rod. The dish is inverted and incubated at 25 ℃ for 2~3 d (culturing time of *Heterotrophic bacteria* will depend on growth condition of bacteria; persons from FIO will check the growth regularly and make relevant records), and then it is taken out for counting the number of colonies.

2216E media:

Peptone 5 g, yeast extract 1 g, ferric phosphate 0.1 g, agar 20 g, seawater 1000 mL, pH7.5

b) *Vibrio cholera*: filter membrane method (GB17378.6-2007)

In the previous BWTS on-board testing, no vibrio has ever been detected in the treated seawater using the plate method. So we filtered the seawater this time. TCBS selective medium is chosen to examine the amount of *vibrio* spp. . 5 mL water sample is filtered through 0.22 μm sterilized acetates fiber membrane. The membrane is then inoculated in TCBS medium. The dish is inverted and incubated at 37 ℃ for 24 h. After the inoculation, the dish is incubated for a certain time under optimal conditions. Then the numbers of the yellow colonies that have the characteristic of *vibrio* spp. infection are counted. If there is any vibrio-like clone on the agar culture plates for samples treated by ballast water treatment systems (BWTS), we identify the clone by a method of monoclonal antibody agglutination. Specifically, the suspected clone is selected and mixed with the monoclonal antibody against *Vibrio cholerae* (serotypes 01and 0139) on a glass slide. If there is agglutination stimulated by antigen-antibody reaction, the tested clone is confirmed to be *vibrio cholera*. If none clones are reacted, we decide there is no *vibrio cholera*.

TCBS media

Yeast extract 5.0 g, peptone 10 g, sodium thiosulphate 10 g, sodium citrate 10 g, ox-bile powder 5 g, glycocholate sodium 3 g, socrose 20 g, ferric citrate 1 g, bromothymol blue 0.04 g, thymol blue 0.04 g, agar 18 g, seawater 1000 mL, pH 8.6

c) *Escherichia coli*: filter membrane method (GB17378.6-2007)

10 mL water sample is filtered through 0.22 μm sterilized acetates fiber membrane. The membrane is then inoculated in M-TEC medium. The dish is inverted and incubated at 37 ℃ for 0.5 h. Then it is transferred to another incubator at 44 ℃ for a continuous cultivation for 24 h. The Escherichia coli colonies on the membrane are then counted and identified, and the number of Escherichia coli per 100mL sea water is

then calculated.

M-TEC media

Peptone 5 g, yeast powder 3 g, lactose 10 g, dipotassium phosphate 3.3 g, monopotassium phosphate 1.0 g, sodium dodecyl sulfate 0.2 g, deoxysoudium cholate 0.1 g, bromocresol purple 0.08 g, bromphenol red 0.08 g, agar 18 g, seawater 1000 mL, pH 7.4

d) *Intestinal Enterococci*: filter membrane method

30 mL water sample is filtered through 0.22 μm sterilized acetates fiber membrane. The membrane is then inoculated in PSE medium. The dish is inverted and incubated at 37 ℃ for 48 h. Then the numbers of the colonies that have the characteristics of *Intestinal Enterococci* are counted.

PSE media

Peptone 20.0 g, yeast extract 5.0 g, bile (specially for bacteriology) 10.0 g, sodium citrate, esculin 1.0 g, ferric ammonium citrate 0.5 g, sodium azide (NaN_3) 0.25 g, agar 18.0 g, seawater 1000 mL, pH 7.4

Details of testing methods are shown in Table 1:

Table 3-1 Testing Methods

Parameter	**Pre-treatment**	**State**	**Stored condition**	**Maximum holding time**	**Instrument**
Temperature	Immediately determined	/	/	/	Multi-parameter probe
Salinity	Immediately determined	/	/	/	Multi-parameter probe
TSS	Directly filtered	Filtered membrane	-20 ℃	72 h	Electronic balance
POC	Directly filtered	Filtered membrane	-20 ℃	72 h	Elementar Vario ELIII
>50 μm organisms	Visual count and identification on ship board	Filtered water	4-6 ℃	6 h	stereo microscope
10~50 μm organisms	**For raw water samples:** Lugol's -fixed count and identification on board ship;	Filtered water	ambient temperature	24 h	Inverted microscope
	For treated and control samples: FDA-PI staining in Qingdao	Filtered water	4-6℃	24 h	Inverted fluorescence microscope
	MPN method in Qingdao	Water from sampling points directly	4-6 ℃	24 h	Tunner Designs Fluorometer and microscope
microbes	**Pre-treatment**	**State**	**Culture condition**	**Culture time**	**Instrument**
bacteria	Immediately inoculated within 2 hours after sampling	Plate method	25 ℃	2~3 d*	incubator

Vibrio cholera	Immediately inoculated within 2 hours after sampling	Filter membrane method	37 ℃	24 h	incubator
Escherichia coli	Immediately inoculated within 2 hours after sampling	Filter membrane method	37 ℃; 44 ℃	37℃: 0.5 h; 44 ℃: 24 h	incubator
Intestinal *enterococci*	Immediately inoculated within 2 hours after sampling	Filter membrane method	37 ℃	24~48 h	incubator

4. Working Principles

1) Specifications for oceanographic survey Part 5: Marine Chemical survey (GB/T12763.5-2007,in Chinese).
2) Specifications for oceanographic survey Part 6: Marine biological survey(GB/T12763.6-2007,in Chinese).
3) Specifications for oceanographic monitoring Part 4: Water Quality monitoring and analysis (GB17378.4-2007,in Chinese).
4) Specifications for oceanographic monitoring Part 7: Ecological survey for offshore pollution and biological monitoring (GB17378.7-2007, in Chinese).
5) Hallegraeff, Anderson and Cembella. Manual on Harmful Marine Microalgae. Unesco 2004 and 2008.
6) The Marine Environment Protection Committee, 2008. Resolution MEPC.174(58).Guidelines for Approval of Ballast Water Management System(G8).
7) Sub Committee on Bulk Liquids and Gases IMO 15th session Agenda item 5. 12/2010.Development of guidelines and other documents for uniform implementation of the 2004 BWM convention ,Additional guidance on indicative analysis。
8) Water quality Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method British Standard ISO 7899-2:2000.
9）Water quality - Detection and enumeration of *Escherichia coli* and coliform bacteria .ISO 9308-1-2000.
10）An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. Journal of Histochemistry & Cytochemistry. Vol.33, No 1,PP.77-79.

5 Quality Control

5.1 Quality System

Perform as “Quality assurance project Plan of sample analysis in the process of the testing for Seascape®-BWMS marine ballast water purification treatment”.

5.2 Quality Control

5.2.1 Measures of Quality Assurance

5.2.1.1 Measures of quality assurance for sampling in field

During the shipboard tests all the samples are collected in field and subdivided into appropriate bottles with labels. These containers and/or bottles from 0.1 L to 1000 L are used for sampling and/or storage. Samples were taken continuously and evenly during the whole process of filling or emptying the ballast water tanks. To prevent contamination, the bottles should be washed by hydrochloric acid, cleaned by pure water, and washed by seawater before sampling on the scene. These containers are thoroughly rinsed or sterilized under high pressure. Each medium for the human pathogens should be prepared in the laboratory and sterilized on site. The organism ≥ 50μm are filtrated using the 50 μm size mesh net on intake or discharge points (1 m^3 of influent water and discharge water each), concentrated to 100 mL-200 mL and put into a new small bottle with label.

Samples for the 10 μm-50 μm size are collected as whole undisturbed samples. These samples are then filtered sequentially through a 50 μm and a 10 μm mesh net and concentrated to 100 mL-200 mL and put into a new small bottle with label.

5.2.1.2 Measures of quality assurance for preservation and transportation of samples

Anti-contamination measures should be taken during filtering and subdividing samples. Wear gloves when testing POC, TSS and organisms. Samples that cannot be immediately analyzed (such as TSS and POC) should be preserved with dry ice after pre-treatment and put in the refrigerator (if no dry ice we use ice bag). Samples for planktons fixed with Lugol's iodine solution (for 10 μm ~50 μm) and/or formaldehyde solution (≥50 μm) and transported to the laboratory at ambient temperature. Organism samples for discharge water of treatment and control tank should not be fixed and transported to the laboratory at about 4℃.

All the samples for biology analysis should be finished in 24 hours.

5.2.2 Quality Control

5.2.2.1 Quality control for testing process.

All kinds of analysis instruments should meet the project requirements.

Before testing, check the samples to make sure that the internal and external labels should be the same as recorded on site.

All the analysis instruments should be in normal working state after testing.

If there are some abnormal results, analyze the reasons in time to get reasonable and analytical conclusions; if necessary, analyze and test the samples once again.

All the analyzing personnel have the Marine environment monitoring on-job certificates except for graduate students. Although graduate students have no on-job certificates, they are all trained through specialized tests.

5.2.2.2 Quality control for detecting process

All the analyzing personnel should be clear about their working and quality responsibilities, respectively.

Check if the instruments are normal; after installing the instruments, check and calibrate them again; make records at the same time.

All personnel should operate the instruments on the basis of the standards that meet relevant requirements.

All the testing instruments should be in normal working state after testing.

If the testing process is interrupted or some operations need to be changed, report to relevant leaders and get necessary approvals.

5.2.2.3 Control of analysis instruments

All the instruments should be checked by national legal institutions and make sure that they are used in the effective period. Calibrate the instruments before using.

5.2.3 Original Records

1) As truthful records of the testing results, original records should not be allowed to change or delete at one's own sake.

2) The format of original records should be uniform; it is not allowed to record the data with a pencil; testing and calibrating personnel should sign their name on the records.

3) The significant digit of data and data processing are required to conform to the standard in GB/T-12763-2008 and the regulation of Marine Monitoring Specification.

6 Technical and Organization Measures

6.1 Resource

6.1 Instrument Resource

Table 6-1 Instrument resources

Name	Specification	Precision	Producer
Filter net	net mouth diameter 37cm	silk mesh 50 μm (diagonal dimension)	America
Filter net	net mouth diameter 25cm	silk mesh 10 μm (diagonal dimension)	America
Electronic balance	ME614S	0～610g, 0.1mg	Germany
CHN elemental analyzer	Elementar Vario ELIII	±0.2%	Germany
Fluorescence microscope	Nikon EC501	100—1000×	Japan
Inverted fluorescence microscope	Nikon TE2000-U	40—400×	Japan

Inverted microscope	Nikon TS100	40—400×	Japan
Stereo microscope	Leica S6E	10—200×	Germany
Filter device	250mL，500mL		China
Multi-parameter analyzer	YSI6600	Temperature 0.0-100.0, ±0.1°C Salinity 0.00—80.00, ±0.5%	Switzerland

6.2 Working group

Project leader: Ruixiang Li, professor, major of marine biology, engaged in Marine phytoplanton taxonomy, biology and ecology and harmful algal bloom, and has on-job certificates for environmental impact assessment of construction projects, sea area use demonstration, oceanographic ecological survey, etc. She has carried out Natural Science Foundation of China and other special research projects. The following table is about group profile of the project.

Table6-2 group profile of the project

Name	Education background	Major	Job title	Responsibility in this project	On-job certificate
Ruixiang Li	Undergraduate	Marine biology	Prof.	Leader	Refer to QAPP
Ping Liu	Undergraduate	Marine biology	Assistant researcher	Zooplankton	Refer to QAPP
Yan Li	Master	Marine biology	Associate researcher	Phytoplankton	Refer to QAPP
Jinxing Zhang	Undergraduate	Marine biology	Prof.	Microorganism	Refer to QAPP
Baodong Wang	Doctor	Marine chemistry	Prof.	Chemistry	Refer to QAPP
Linping Xie	Master	Marine chemistry	Assistant researcher	Chemistry	Refer to QAPP
Lingyun Qu	Doctor	Marine biology	Prof.	Microorganism	Refer to QAPP
Ping Sun	Master	Marine biology	Assistant researcher	Phytoplankton	Refer to QAPP
Xiao Wang	Doctor	Marine biology	Doctor	Zooplankton	Refer to QAPP
Bin Xia	Undergraduate	Marine biology	Senior engineer	Chlorophyll	Refer to QAPP

7 Submitted documents

7.1 Analysis report

7.2 Data information